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44We CLAIMS

1. A kallikrein inhibiting protein which comprises a non-naturally occurring Kunitz domain, wherein, at each of the residues of said domain corresponding to the below identified residues of BPTI, one of the following allowed amino acids is found:

BPTI
residue #Allowed Amino Acid

| | |
|----|--|
| 10 | Asp, Glu, Ala, Gly, Ser, Thr |
| 11 | Asp, Gly, Ser, Val, Glu, Leu, Met, Asn, Ile, Ala, Thr |
| 12 | Gly, and, if residue 14 or 38 is not Cys, any conservative or semi-conservative substitution for a "normal" conformation Gly as defined in Table 9 |
| 13 | Arg, His, Pro, Asn, Ser, Thr, Ala, Gly, Lys, Gln |
| 14 | Cys, and, if residue 38 is not Cys, any conservative or semi-conservative substitution for Cys |
| 15 | Arg, Lys, Ala, Ser, Gly, Met, Asn, Gln |
| 16 | Ala, Gly, Ser, Asp, Asn |
| 17 | Ala, Asn, Ser, Ile, Gly, Val, Gln, Thr |
| 18 | His, Leu, Gln, Ala |
| 19 | Pro, Gln, Leu, Asn, Ile |
| 20 | Arg, Leu, Ala, Ser, Lys, Gln, Val |

83

70
45

| | |
|----|--|
| 21 | Trp, Phe, Tyr, His, Ile |
| 31 | Glu, Asp, Gln, Asn, Ser, <u>Ala</u> , <u>Val</u> , <u>Leu</u> , <u>Ile</u> , Thr |
| 32 | Glu, Gln, Asp, Asn, Pro, Thr, Leu, Ser, Ala, Gly, Val |
| 33 | Phe, Tyr |
| 34 | Ser, Thr, Ile, Val, Ala, Asn, Gly, Leu |
| 35 | Tyr, Trp, Phe |
| 36 | Gly, Ser, Ala |
| 37 | Gly, and, if residue 14 or 38 is not Cys, any conservative or semi-conservative substitution for a "normal" conformation Gly as defined in Table 9 |
| 38 | Cys, and, if residue 14 is not Cys, any conservative or semi- conservative substitution for Cys |
| 39 | Gly, Glu, Ala, Ser, Asp. |

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2. A kallikrein inhibiting protein which comprises a non-naturally occurring Kunitz domain, wherein, at each of the residues corresponding to the below identified residues, one of the following allowed amino acids is found:

| <u>BPTI</u> <u>residue #</u> | <u>Allowed Amino Acid</u> |
|---------------------------------|---|
| 10 | Asp, Glu, Ala, Gly, Ser, Thr |
| 11 | Asp, Gly, Ser, Val, Glu, Leu, Met |
| 12 | Gly, and, if residue 14 or 38 is not Cys, <u>or</u> any conservative |

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84

71
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semi-conservative substitution
for a "normal" conformation Gly
as defined in Table 9

- 13 Arg, His, Pro, Asn, Ser
- 14 Cys, and, if residue 38 is not
Cys, any conservative or semi-
conservative substitution for
Cys
- 15 Arg, Lys
- 16 Ala, Gly
- 17 Ala, Asn, Ser, Ile
- 18 His, Leu, Gln
- 19 Pro, Gln, Leu
- 20 Arg, Leu, Ala, Ser, Lys, Gln,
Val
- 21 Trp, Phe
- 31 Glu
- 32 Glu, Gln
- 33 Phe
- 34 Ser, Thr, Ile
- 35 Tyr
- 36 Gly, Ser, Ala
- 37 Gly, and, if residue 14 or 38
not Cys, any conservative or
semi-conservative substitution
for a "normal" conformation Gly
as defined in Table 9
- 38 Cys, and, if residue
corresponding to position 14 is
not Cys, any conservative or
semi-conservative substitution
for Cys

85

72

47

39

Gly, Glu, Ala.

6 3. The protein of claim 2 wherein, the Kunitz domain is further characterized as follows:

10960X

| <u>BPTI</u> <u>Residue No.</u> | <u>Allowed Residue</u> |
|-----------------------------------|------------------------|
| 10 | Asp, Glu |
| 11 | Asp, Gly, Ser, Val |
| 12 | Gly |
| 14 | Cys |
| 20 | Arg |
| 36 | Gly |
| 37 | Gly |
| 38 | Cys. |

4. A plasma kallikrein inhibiting protein which comprises a sequence that is substantially homologous to a reference sequence selected from the group consisting of

Sub B3

KKII/3 #1, KKII/3 #2, KKII/3 #3, KKII/3 #4, KKII/3 #5, KKII/3 #6, KKII/3 #7, KKII/3 #8, KKII/4 #9, KKII/3 #10, KK2/#11, KK2/#13, KK2/#1, KK2/#2, KK2/#3, KK2/#4, KK2/#6, KK2/#7, KK2/#8, KK2/#9, KK2/#10, KK2/#12, AND KK2con1 as defined in Table 2.

C 2 5. A method of ~~preventing~~ or treating a disorder attributable to excessive kallikrein activity which comprises administering, to a human or animal subject who would benefit therefrom, a kallikrein-inhibitory amount of the protein of ^{claim 1} ~~any of claims 1-4~~.

Sub B4 6. A method of assaying for kallikrein which comprises providing

73
48

claim 1
A the protein of ~~any of claims 1-4~~ in labeled or insolubilized form, and determining whether a complex of said protein and the kallikrein in a sample is formed.

Sub B4 cont.
7. A method of purifying kallikrein from a mixture which comprises providing the protein of *claim 1* ~~any of claims 1-4~~ in insolubilized form, and contacting the mixture with said insolubilized protein or analogue so that kallikrein in the mixture is bound.

Add A1

Add B2